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[Article]

Classification of Larval and Adult Delta Smelt to Nursery Areas by Use of Trace Elemental Fingerprinting

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Abstract.—Different environmental conditions among habitats may generate unique elemental patterns within fish otoliths that can be used to trace the life history as well as the potential natal origin of migratory species. We investigated the use of trace elements in otoliths as natural tags for determining the natal origins of larval and adult delta smelt *Hypomesus transpacificus* within a single estuary. Larval fish were collected at potential natal sites within the San Francisco Bay Estuary—the North, Central, South, and West Delta areas, Suisun Bay, and Napa River—during May–June 1999; adults were collected in November 1999 throughout Suisun Bay. Using laser ablation inductively coupled plasma mass spectrometry, we assayed trace elements from core (natal) regions of the otolith (Sr:Ca, Mg:Ca, and Ba:Ca ratios). Linear discriminant function analysis (LDFA) was 90.9–100% successful at classifying larval fish to their natal habitats (Napa River, Sacramento River, and Delta). Adults of unknown natal origin were assigned to their natal regions using the larval fingerprints from LDFA and a maximum likelihood mixed-stock approach. For the 1999 year-class, we determined that a majority of the population originated from the Delta (77–79%) and a small but significant proportion of the population originated from the Napa River (16–18%) and Suisun Bay (4–8%). These data highlight the value of trace elements as natural tags for determining the natal origins of young fish and the relative contribution of different habitats to the adult population within a single estuary.

Recent precipitous declines in delta smelt *Hypomesus transpacificus* and the resulting petition to change the species' status from threatened to endangered under the California Endangered Species Act and U.S. Endangered Species Act have resulted in substantial concern regarding the overall condition of the San Francisco Bay Estuary ecosystem (Moyle et al. 1992; Sweetnam and Stevens 1993; USFWS 1995; CALFED 2000; Armor et al. 2005; Miller et al. 2006). Several factors operating at multiple levels of the system have been identified as potential causes for the population decline. Some factors, such as entrainment in large freshwater export facilities in the South Delta, operate more locally in regions of the estuary to impact the population. However, others, such as declines in overall food supply, can affect the entire population (Bennett 2005). To adequately assess the various factors that influence the delta smelt population, we must first develop the tools that will allow researchers to disentangle localized effects from those that can impact the entire population.

Delta smelt are primarily annual and semianadromous; they live within the low-salinity region of the San Francisco Estuary (SFE) but migrate to freshwater to spawn (Moyle 2002). The SFE is characterized by a significant amount of freshwater flow from several major rivers that feed directly into the estuary over a distance of 60 km; these rivers include the Sacramento, San Joaquin, Mokelumne, and Napa rivers. Previous studies suggest that delta smelt utilize a life history strategy that spreads the risk of catastrophic mortality by opportunistically spawning in favorable habitats throughout the freshwater regions of the estuary (Bennett 2005). Particle tracking models have shown that the larvae rear in freshwater habitats of the lower Sacramento and San Joaquin rivers for approximately 30 d (Sommer et al. 2005), then drift downstream to the low-salinity zone as juveniles and eventually recruit to the adult life stage during the fall (Bennett 2005). Because delta smelt exploit freshwater habitats throughout the SFE for spawning, considerable geographic variability exists within the population. Furthermore, regional water quality monitoring programs have found considerable variability in the chemical composition of the various rivers contributing

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to the SFE (SFEI 2001). Given these geographical chemical differences, the application of otolith trace element chemistry may facilitate the identification of these natal habitats for successful recruits.

Otoliths are formed by an alternating daily deposition of calcium carbonate and protein layers. Given the biogenic structure of otoliths, any elements incorporated into the otolith during formation cannot be reabsorbed by the body during periods of stress (Campana and Neilson 1985). As the layers form, ions in the water surrounding the fish are integrated into the calcium matrix by substitution for the calcium or as a result of binding with proteins (Dove and Kingsford 1998). Elemental uptake into the otolith occurs through a complex pathway in which trace elements are differentially absorbed into the bloodstream, endolymph, and the calcium carbonate matrix (Kalish 1991; Campana 1999). Concentrations of trace elements within otoliths may reflect the elemental concentrations in the water (Bath et al. 2000; Kraus and Secor 2004). However, several studies have described factors that can modify this relationship, including temperature (Kalish 1991; Townsend et al. 1992; Martin et al. 2004; Martin and Thorrold 2005), salinity (Fowler et al. 1995a, 1995b; Secor et al. 1995; Elsdon and Gillanders 2005), growth rate (Sadavoy and Severin 1994), age (Radtke and Targett 1984), and diet (Gallahar and Kingsford 1996). Thus, environmental variability and biological processes can result in unique elemental signatures for fish reared in different habitats (see Elsdon and Gillanders 2003 for a review).

Otolith trace elemental chemistry has been applied to many ecological and fisheries issues, such as the identification of stock structure, description of migration histories, and determination of natal origins for a number of species (Edmonds et al. 1989, 1991, 1992; Campana et al. 1994; Secor et al. 1995, 2001; Secor 1999; Rooker et al. 2003; Kraus and Secor 2005). For example, on the U.S. East Coast, Thorrold et al. (1998b) utilized trace elements to classify juvenile weakfish *Cynoscion regalis* to their specific natal estuaries. These juvenile signatures were then used to identify the estuarine origins for unknown adult weakfish within the same system (Thorrold et al. 2001). Similarly, Gillanders and Kingsford (2000) used trace elements to distinguish estuarine origins of juvenile eastern striped trumpeters *Pelates sexlineatus*, which exploit several different estuaries along the east coast of Australia. Although these studies focused on variability between estuaries, the authors found considerable differences between sites located within these estuaries. To utilize trace elemental signatures as natural tags for natal habitats at small spatial scales, such as sections of a river or estuarine gradient, there must be considerable environmental variability and high retention of larval fish within those habitats (Secor et al. 2001).

We investigated the ability of trace elements to act as natural tags to identify potential natal habitats within a single estuary. The observation that delta smelt larvae remain in freshwater habitats for approximately 30 d prior to estuarine migration minimized the possibility that larvae collected within a particular natal area originated elsewhere (Sommer et al. 2005). We evaluated this tool during a moderately wet year, when delta smelt recruitment was high and spatial distribution was greatest, to fully assess the potential to discriminate all natal regions of the estuary. Our specific goals were to (1) classify larval delta smelt to specific freshwater natal habitats within the SFE using unique chemical signatures from the core (natal) region of the otolith and (2) determine whether larval signatures are useful for ascertaining the natal origins of adult delta smelt.

Methods

Larval delta smelt were collected during May–June 1999 at six sites: Napa River (N); lower Sacramento River near Cache Slough (i.e., North Delta [ND]); Central Delta near the Mokelumne River (CD); South Delta near the San Joaquin River (SD); confluence of the Sacramento and San Joaquin rivers near Sherman Island (i.e., West Delta [WD]); and Montezuma Slough in Suisun Bay (SB; Figure 1). Larval and adult delta smelt collected at each of these sites were examined for mean otolith size and standard length (SL) using analysis of variance (ANOVA; Table 1). Previous genetic studies were unable to find significant genetic variability within the delta smelt population, suggesting larvae collected in different habitats represent distinct geographic cohorts rather than separate populations (Trenham et al. 1998). The above areas were chosen as possible natal areas based on the occurrence of yolk sac larvae at only these sites during the California Department of Fish and Game (CDFG) 20-mm survey (a long-term monitoring program for delta smelt; see CDFG 2004 for details). Subsequently, in November and December 1999, we collected adult delta smelt during the CDFG midwater trawl survey after the downstream migration. Samples processed and utilized in this study represented 16 stations located in four of the sites in November and seven stations in two of the sites in December. The collection sites represented the extent of the adult spatial distribution during these surveys, and the number of samples processed for November and December were 32–34% of all samples collected by CDFG for these surveys; thus, our sample scheme was meant to represent the delta smelt

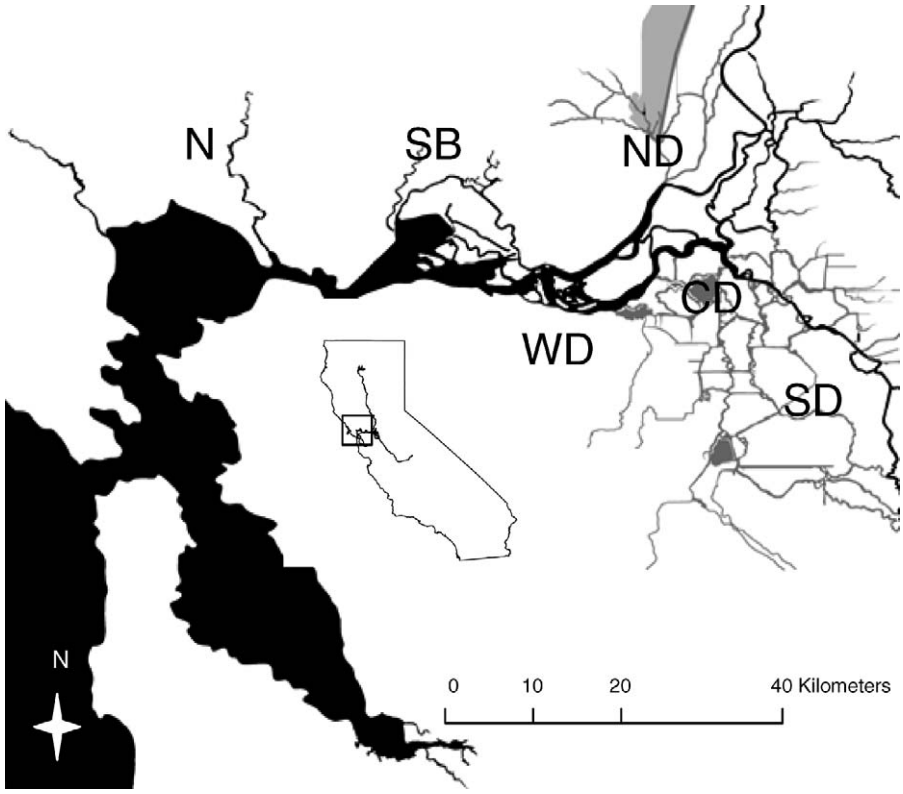

FIGURE 1.—Location of delta smelt natal regions and sampling sites in the San Francisco Bay Estuary (N = Napa River; SB = Suisun Bay–Marsh; WD = West Delta; ND = North Delta; CD = Central Delta; SD = South Delta).

population (www://delta.dfg.ca.gov/midwatertrawl.html; Figure 1). All fish were field preserved in a 95% solution of ethanol according to CDFG protocols and returned to the laboratory. While Sr and Ba concentrations are not influenced by ethanol preservation, the effect on Mg concentrations is unknown. However, all fish in this study were preserved similarly, minimizing the effect of preservation on the otolith chemical fingerprint (Hedges et al. 2004).

TABLE 1.—Summary information for delta smelt collected for trace elemental chemistry comparisons among natal areas in the San Francisco Estuary. Shown are regions where fish were collected, month of collection in 1999 mean (±SE) SL (mm), and mean (±SE) otolith weight (OW; mg).

Sample site	Sample month	*N*	Mean SL	Mean OW
		Larvae		
Napa River	Jun	11	26.5 ± 1.5	0.21 ± 0.05
Suisun Bay	May	8	24.5 ± 1.8	0.19 ± 0.08
North Delta	Jun	21	25.5 ± 2.2	0.20 ± 0.03
West Delta	Jun	8	23.1 ± 2.3	0.19 ± 0.09
Central Delta	May	26	27.2 ± 5.2	0.22 ± 0.10
South Delta	May	11	26.2 ± 2.7	0.21 ± 0.10
		Adults		
Suisun Bay	Nov	20	63.2 ± 6.7	1.21 ± 0.03
North Delta	Nov	18	66.8 ± 6.8	1.31 ± 0.05
Central Delta	Nov	11	61.1 ± 3.6	1.20 ± 0.03
West Delta	Nov	12	58.9 ± 9.4	1.25 ± 0.09
Suisun Bay	Dec	10	62.0 ± 3.3	1.25 ± 0.04
West Delta	Dec	29	63.0 ± 5.3	1.24 ± 0.05

In the laboratory, fish were measured for SL; no correction for shrinkage was made. The sagittal otoliths were removed, weighed on a microbalance, and cleaned in Milli-Q water. Otoliths were then mounted on glass slide, affixed with Crystal bond thermoplastic wax, and polished on both sides with 3-μm lapping film. Lastly, the otoliths were washed with a 1% solution of nitric acid for 5–10 s, rinsed in an ultrasonic water bath for 5 min, and dried under a class-100 laminar flow hood.

Otolith elemental chemistry.—Trace elemental concentrations were analyzed with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS; Agilent 7200a ICP Mass Spectrometer coupled with a New Wave Nd:Y–Al–garnet UP213 laser ablation system) at the Interdisciplinary Center for Plasma Mass Spectrometry, University of California, Davis (UC–Davis). Three replicate spots near the core

of the otoliths were quantified at a spot size of 20 μm (laser pulsing at 5 Hz) for a dwell time of 30 s. This spot size and position with respect to the core resulted in a sample collection of approximately 1 week. Data were collected in a time-resolve mode for a total of 90 s. A background gas signal was collected for 30 s prior to the sample signal. The three elements of interest (^{88}Sr, ^{137}Ba, ^{25}Mg), were background corrected, ratioed to an internal standard (^{45}Ca), and standardized using the National Institute of Standards and Technology (NIST) 612 glass standard. The ratios of calculated element concentrations to the total Ca of the sample (μmol : mol or mmol : mol) were determined based on stoichiometry.

The accuracy of LA-ICP-MS is often evaluated using percent relative standard deviation (%RSD) of a reference standard during experiments (Campana 1997). The accuracy and precision of this technique should be evaluated by examining three main components of the measurement process (see Fallon et al. 1999 for details). First, counting uncertainty (the error associated with the counting statistics) was expressed as one SE of the background-corrected counts on the elements examined, where background counts are similar to detection limits. Second, calibration error was assessed by comparing the mean concentration of all replicate measurements on the NIST 612 standard with the published concentrations of the elements in question, and error was expressed as the percent difference for the concentration of each element relative to the standard published value. Third, error associated with heterogeneity of the standard was calculated as one SD of all replicate spots on the NIST 612 standard. Because the data were collected over several experimental dates, we also assessed experiment reproducibility for each element by calculating the %RSD of all experimental dates of the NIST 612 (Table 2). With this approach, experimental reproducibility and the heterogeneity of the standard would be two components of the %RSD.

Measures of trace element concentrations were statistically analyzed using parametric multivariate techniques. All concentrations of trace elements were standardized to Ca, and data presented as the ratio of trace element to Ca. We used multivariate ANOVA (MANOVA) to test the null hypothesis of no significant differences in trace element ratios among locations with Bonferroni corrections to test differences among natal origins. First, we log transformed the data ($\log[x + 1]$). The assumption of multivariate normality and the equality of the variance–covariance matrices were then assessed by examining each of the univariate variables for normal distribution of errors and homogeneity of variance using residual analysis (Winer et al. 1991). All variables met the assumptions of normality and homogeneity of variances after log transformation.

TABLE 2.—Accuracy, precision, and reproducibility of laser ablation inductively coupled plasma mass spectrometry. All measurements were conducted on NIST 612 glass standard (20-m spots; 30-s dwell).

Error (%)	Element			
	^{25}Mg	^{88}Sr	^{137}Ba	^{55}Mn
Counting uncertainty[a]	0.6	0.3	0.4	0.1
Calibration of standard[b]	0.5	1.2	1.6	2.5
Heterogeneity of standard[c]	4.6	3.5	5.3	2.1
Experiment reproducibility[d]	2.2	4.7	6.4	1.0

[a] SE relative to the background-corrected counts per second using Glitter.
[b] Overall mean concentrations relative to published NIST 612 concentrations.
[c] SD relative to the mean concentration.
[d] Based on SD of multiple dates of measurements on NIST 612 glass standard.

Linear discriminant function analysis (LDFA) was used to determine whether trace element ratios to calcium in otoliths of larval delta smelt reflected differences in natal origins. Using the jackknife procedure of general linear model discriminant function, each sample was removed sequentially from the data set and the discriminant function was calculated from the remaining data. Cross validation analysis was also used to determine the percent of fish accurately classified to natal origin. The larval data set was used as a reference data set in LDFA to identify the adults of unknown natal origin. In addition, the classification of adults back to their natal origins was further assessed using a maximum likelihood mixed-stock analysis approach (integrated stock mixture analysis [ISMA]) as described by Campana et al. (2000). The ISMA model has distinct advantages over the LDFA model: prior probabilities of group membership are known, and the ISMA model is relatively distribution insensitive. However, the ISMA model can be sensitive to small sample sizes. The ANOVA and MANOVA were conducted using SYSTAT 10.0 (SPSS 2000), the LDFA was conducted with MATLAB, and the ISMA was conducted with S+ statistical software.

Results

Larval delta smelt collected from the six hypothesized natal habitats (Figure 1) ranged in size from 17 to 28 mm SL. There were no significant differences in mean length (ANOVA: $df = 5, 79$; mean square [MS] = 2.02; $F = 9.63$; $P = 0.67$) and otolith weight (ANOVA: $df = 4, 26$; $MS = 0.019$; $F = 12.74$; $P \leq 0.001$) among natal habitats; therefore, length was not considered further in statistical analyses (Table 1). For the

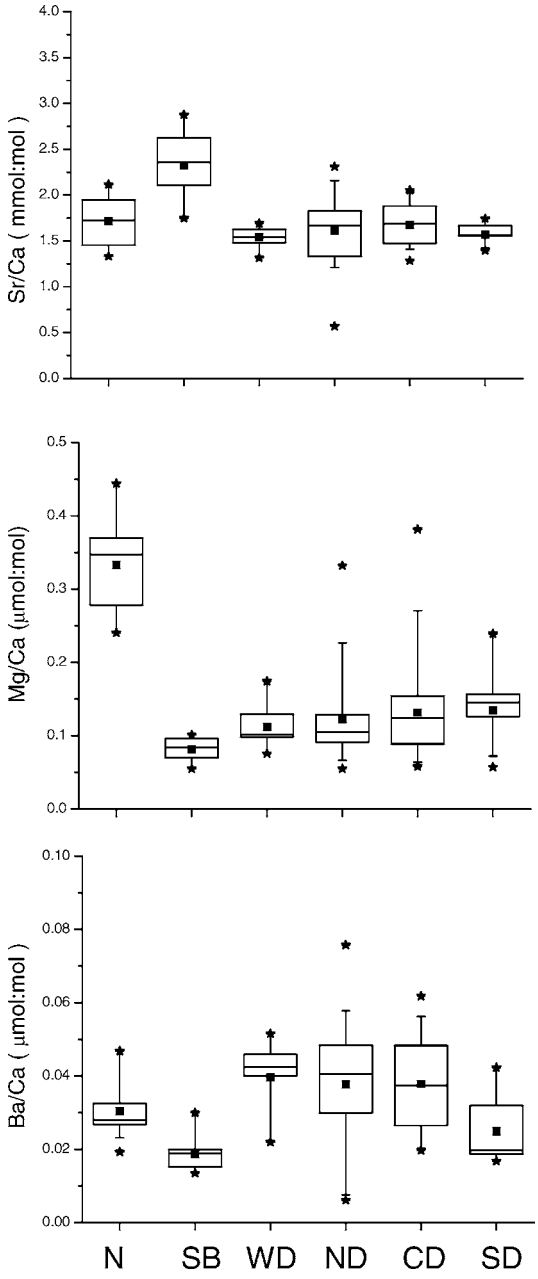

FIGURE 2.—Box plot of mean element concentrations (Sr, Mg, and Ba) expressed as ratios to Ca in the otoliths of larval delta smelt collected from natal regions of the upper San Francisco Bay Estuary in 1999. Natal region codes (*x*-axis) are defined in Figure 1.

November survey, there were differences in adult SL (ANOVA: df = 3, 53; MS = 162.47; $F = 3.22$; $P = 0.03$) and otolith weight (ANOVA: df = 3, 53; MS = 133.17; $F = 3.10$; $P = 0.04$) associated with capture locations; however, for the December survey no differences were found for SL (ANOVA: df = 1, 37; MS = 7.96; $F = 0.331$; $P = 0.568$) or otolith weight (ANOVA: df = 1, 37; MS = 6.35; $F = 0.435$; $P = 0.423$). Overall, there were no significant differences found for SLs of microchemistry subsamples and SLs of all fish collected during the surveys (November: $t = -1.58$, $P = 0.118$; December: $t = -1.77$, $P = 0.09$).

A difference in trace element chemistry among three of the six natal sites suggests the existence of distinct geographic cohorts within the population (MANOVA: df = 20, 84; $F = 1.436$; $P = 0.0036$). The Sr:Ca ratios were higher at Suisun Bay than at the Delta sites or the Napa River, while Mg:Ca ratios were higher at the Napa River site than at all other sites. The Napa River and Suisun Bay had lower Ba:Ca ratios than those of the West, Central, and North Delta sites but not the South Delta site (Figure 2; Table 3). Because of the lack of differences among Delta sites (the exception being the South Delta for Ba:Ca), we combined all Delta sites for further analyses.

Classification success as determined by jackknifed cross validation for larval delta smelt was high: 90.9% for the Napa River, 100% for Suisun Bay, and 95.5% for the combined Delta sites (Table 4). Elemental signatures derived from the canonical discriminant functions exhibited clear separation of the Napa River cohort from the Suisun Bay and Delta cohorts along the first canonical variate, which was strongly, positively influenced by Mg:Ca concentrations and negatively influenced by Ba:Ca. The Suisun Bay cohort was readably distinguishable from the Napa River and Delta cohorts along canonical variate 2, which was influenced by high Sr:Ca concentrations (Figure 3A).

Discriminant functions from the larval reference data set were used to calculate individual canonical scores and identify unknown adult natal origins. Adult canonical scores exhibited a similar separation of natal sites along the first two canonicate variables; the Napa River cohort was strongly, positively associated with canonical variate 1 and Suisun Bay was negatively associated with variate 2 (Figure 3B). Of the 100 unknown adults, 18 were identified by LDFA as belonging to the Napa River cohort, 4 were classified as belonging to the Suisun Bay cohort, and 78 were designated as belonging to the Delta cohort. The significance of the adult classification was assessed using the Mahalanobis distance-squared method and was 0.056; only two individuals from the Napa River group were incorrectly classified to the Delta group. The ISMA model produced results similar to those of the LDFA: of the 100 adult samples, 16 were identified as belonging to the Napa River cohort, 8 were identified as belonging to the Suisun Bay cohort, and

TABLE 3.—Analysis of variance results for three trace elements (expressed as ratios to Ca) in otoliths of larval delta smelt collected within six natal regions of the San Francisco BayEstuary in 1999. Multiple comparisons were conducted with Bonferroni corrections. Natal region codes are defined in Figure 1 (ns = not significant; SS = sum of squares; MS = mean square).

							Pairwise comparison (Bonferroni probability) by region					
Source	df	SS	MS	*F*	*P*		N	SB	ND	CD	WD	SD
Mg:Ca						N						
Origin	5	0.473	0.095	36.49	<0.001	SB	<0.001					
Error	79	0.205	0.003			ND	<0.001	ns				
						CD	<0.001	ns	ns			
						WD	<0.001	ns	ns	ns		
						SD	<0.001	ns	ns	ns	ns	
Sr:Ca						N						
Origin	5	3.971	0.794	8.54	<0.001	SB	0.001					
Error	79	7.349	0.093			ND	ns	<0.001				
						CD	ns	<0.001	ns			
						WD	ns	<0.001	ns	ns		
						SD	ns	<0.001	ns	ns	ns	
BA:Ca						N						
Origin	5	0.004	0.0008	5.12	0.0004	SB	ns					
Error	79	0.012	0.0002			ND	ns	0.005				
						CD	ns	0.003	ns			
						WD	ns	0.015	ns	ns		
						SD	ns	ns	ns	ns	ns	

76 were classified as belonging to the Delta cohort (Table 5).

Discussion

If trace elements are to be used as markers for natal habitats, these signatures must be distinct for each specific geographic location. In this study, larval delta smelt otoliths did exhibit distinct variations in elemental signatures among three of the six sampled natal sites, suggesting identification of natal sites at larger geographical scales is possible for delta smelt. For example, Napa River delta smelt displayed considerable differences in elemental signatures from fish originating in the Delta as well as Suisun Bay. This resulted in successful classification for each of these three natal areas and verified that the Napa River functions as natal habitat for delta smelt, thereby contributing fish to the adult population. Thus, otolith elemental signatures effectively identified specific natal origins for adult delta smelt and classified differences among geographically distinct cohorts.

Numerous studies have documented the ability of trace elements to accurately classify fish to different natal estuaries for marine and freshwater spawning fish at large geographical scales (Thorrold et al. 1998b; Gillanders and Kingsford 2000). However, while trace elements were useful for distinguishing natal habitats at large geographic scales (e.g., Napa River and Delta > 20 km), smaller spatial scales (e.g., sites within the Delta < 10 km) remained indistinguishable and resulted in inaccurate classifications for natal origin. Two potential mechanisms for these inaccurate classifications are (1) the mixing of several rivers within the Delta, resulting in a homogenous chemical fingerprint or (2) the high dispersal of planktonic larvae away from natal habitats, resulting in highly variable otolith signatures at small spatial scales (e.g., North Delta-born fish occurring in the South Delta catch). Considerable variability among individual trace element signatures within the Delta sites suggests that the inaccurate classification of delta smelt to their natal origins resulted from a high dispersal of the larvae away from their natal habitats.

Studies conducted at similar spatial scales have also found similar results in which some species exhibited significant differences in trace elemental chemistry (Thorrold et al. 1998a; Gillanders and Kingsford 2000). Another study found little variation between sites (Thorrold et al. 1997). Significant differences in trace element signatures were found at sites within rivers or estuaries when dispersal was limited. Thus, the scale at which trace elements can be utilized depends predom-

TABLE 4.—Jackknifed cross-validation accuracy (%) for classification of larval delta smelt to natal region within the San Francisco Bay Estuary, as determined by otolith trace element ratios (Ba:Ca, Sr:Ca, and Mg:Ca).

	Natal region		
Collection site	Napa River	Suisun Bay	Delta
Napa River ($n = 11$)	90.9	0	4.5
Suisun Bay ($n = 8$)	0	100	0
Delta ($n = 66$)	9.1	0	95.5

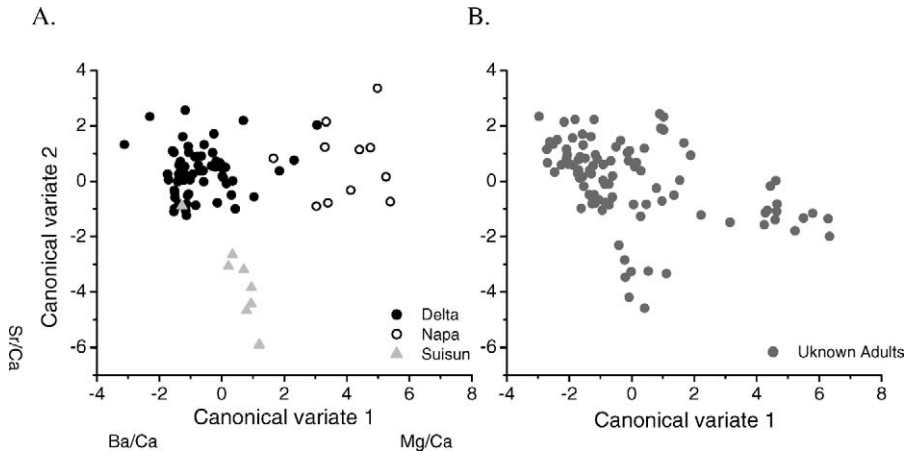

FIGURE 3.—Canonical variate plot summarizing variations in trace element signatures (Sr:Ca, Mg:Ca, and Ba:Ca ratios) within the otoliths of **(A)** larval and **(B)** adult delta smelt collected from the San Francisco Bay Estuary in 1999.

inantly on the early life history strategy of a species (e.g., dispersal characteristics), environmental variability, and the geographic scales at which different natal habitats occur.

The discovery of adult fish originating from the Napa River has important implications for understanding the population ecology of delta smelt. Previous conceptual models presented the Napa River as marginal nursery habitat for delta smelt, acting as a potential sink to the adult population (Bennett 2005). Due to the complex geography of the system, the Napa River is usually limited in its accessibility to delta smelt. The Napa River is only available to delta smelt when outflow conditions from the Sacramento and San Joaquin rivers are sufficient to connect the low-salinity region of the estuary to the Napa River (e.g., 1998–1999). Furthermore, delta smelt are sensitive to salinities above 12‰ and frequently avoid salinities greater than 5‰ (Swanson et al. 2000). During summer low-flow conditions, the low-salinity zone retreats into the Delta, disconnecting fish rearing in the low-salinity region of the Napa River from the rest of the estuary. Thus, delta smelt can become isolated in the Napa River. During the fall, salinities in the Napa River estuary often reach 30–32‰, which is well above the salinity tolerance of delta smelt. Delta smelt residing in the Napa River must migrate across the low-salinity front to reconnect with the rest of the population. Despite the limited accessibility of the Napa River, our study clearly demonstrates that delta smelt can utilize the Napa River as natal habitat and that recruits successfully leave the river to join the adult population in Suisun Bay and the Delta. For the restoration of this endangered species, outflow practices within the Napa River and Sacramento–San Joaquin River watersheds must take into consideration all of the potential natal habitats used by delta smelt. In addition, the identification of Suisun Bay fish with elevated Sr:Ca ratios suggests that delta smelt can spawn and rear in low-salinity waters, which is counter to the current knowledge that delta smelt only spawn in freshwater habitats (Moyle et al. 1992). However, elevated Sr:Ca ratios may also result from many other confounding factors, such as temperature and growth. During this study, temperature and growth differences were not evident among the natal habitats. In addition, a previous study conducted in the low-salinity zone found relatively high numbers of delta smelt larvae in these habitats (Bennett et al. 2002). Thus, delta smelt are likely to use low-salinity waters as larval rearing habitat.

The discovery that the delta smelt can utilize several different river systems as well as low-salinity zone habitats provides evidence regarding their ability to spread progeny throughout the estuary, which may protect delta smelt from catastrophic mortality at locations within the estuary where anthropogenic impacts may be significant. The 1999 delta smelt year-

TABLE 5.—Percent contribution of three natal areas to the adult delta smelt population in the San Francisco Bay Estuary, 1999, based on results from linear discriminant function analysis (LDFA) and integrated stock mixture analysis (ISMA) of otolith trace elementratios (Ba:Ca, Sr: Ca, and Mg:Ca).

Origin	LDFA	ISMA
Napa River	18	16
Suisun Bay	4	8
Delta	79	77

class had a record number of fish entrained in freshwater export facilities; however, the adult population abundance was one of the highest in recent years (Bennett 2005). Thus, the occurrence of fish at other natal sites (Napa River and Suisun Bay) may have bolstered the population against catastrophic mortality. However, more work is needed to understand how different river habitats may contribute to the population during different environmental conditions, namely freshwater outflow.

Conclusions

This study demonstrated the usefulness of trace elements as natural tags for distinguishing the natal origins of the endangered delta smelt. More importantly, using otolith elemental signatures, we were able to accurately classify adult fish back to their natal habitats. Furthermore, we discovered that fish originating from the Napa River (previously considered a "sink") did, in fact, contribute to the adult population and that delta smelt can use low-salinity habitats for spawning and larval rearing. This has important implications for restoration of threatened native fish populations and the management of freshwater outflow. Spatial resolution was poor for sites within the Delta, demonstrating the dependence of environmental variability, dispersal abilities, and geographic scale in these types of studies. Recently, Sr isotopes have provided greater spatial resolution of natal rivers for Chinook salmon *Oncorhynchus tshawytscha* (Ingram and Weber 1999). Future studies will incorporate the use of Sr isotope ratios to further elucidate patterns within the Delta region. Ultimately, the ability to determine which habitats contribute to the sustainability of protected or managed species with the use of otolith chemistry should prove invaluable for many applications in fisheries research.

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References

Armor, C., R. Baxter, B. Bennett, M. Chotkowski, P. Coulston, D. Denton, B. Herbold, W. Kimmerer, K. Larsen, M. Nobriga, K. Rose, T. Sommer, and M. Stacey. 2005. Interagency Ecological Program synthesis of 2005 work to evaluate the pelagic organism decline (POD) in the upper San Francisco Estuary. Interagency Ecological Program, Sacramento, California. Available: www.science.calwater.ca.gov/pdf/workshops/POD/CDFG_POD_2005_POD_Synthesis_Report_v5b.pdf.

Bath, G. E., S. R. Thorrold, C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. Geochimica et Cosmochimica Acta 64:1705–1714.

Bennett, W. A. 2005. The population ecology of delta smelt in the San Francisco Estuary. San Francisco Estuary and Watershed Science 3(2):1–71.

Bennett, W. A., W. J. Kimmerer, and J. Burau. 2002. Plasticity in vertical migration by native and exotic estuarine fishes in a dynamic low-salinity zone. Limnology and Oceanography 47:1496–1507.

CALFED. 2000. Strategic plan for ecosystem restoration. CALFED Bay–Delta Program, Sacramento, California. Available: www.delta.dfg.ca.gov/erp/docs/reports_docs/erpp_vol.3.pdf.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms, and applications. Marine Ecology Progress Series 188:263–297.

Campana, S. E., G. A. Chouinard, J. M. Hanson, A. Frechet, and J. Brattey. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fisheries Research (Amsterdam) 46:343–357.

Campana, S. E., A. J. Fowler, and C. M. Jones. 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 51:1942–1950.

Campana, S. E., and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42:1014–1032.

Campana, S., S. Thorrold, C. Jones, D. Gunther, M. Tubrett, H. Longerich, S. Jackson, N. Halden, J. Kalish, P. Piccoli, H. De Pontual, H. Troadec, J. Panfili, D. Secor, K. Severin, S. S. Sie, R. Thresher, W. Teesdale, and J. Campbell. 1997. Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. Canadian Journal of Fisheries and Aquatic Sciences 54:2068–2079. Available: www.delta.dfg.ca.gov/data/20mm.

CDFG (California Department of Fish and Game). 2004. The 20 mm survey. CDFG.

Dove, S. G., and M. J. Kingsford. 1998. Use of otoliths and eye lenses for measuring trace-metal incorporation in fishes: a bio-geographic study. Marine Biology 130:377–387.

Edmonds, J. S., N. Caputi, and C. M. Jones. 1991. Stock discrimination by trace element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. Australian Journal of Marine and Freshwater Research 42:383–389.

Edmonds, J. S., R. C. J. Lenanton, N. Caputi, and M. Morita. 1992. Trace elements in the otoliths of yellow-eye mullet (*Aldrichetta forsteri*) as an aid to stock identification. Fisheries Research (Amsterdam) 13:39–51.

Edmonds, J. S., M. J. Moran, N. Caputi, and M. Morita. 1989. Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in Western Australia waters. Canadian Journal of Fisheries and Aquatic Sciences 46:50–54.

Elsdon, T. S., and B. M. Gillanders. 2003. Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. Reviews in Fish Biology and Fisheries 13:219–235.

Elsdon, T. S., and B. M. Gillanders. 2005. Consistency of patterns between laboratory experiments and field collected fish in otolith chemistry: an example and applications for salinity reconstructions. Marine and Freshwater Research 56:609–617.

Fallon, S. J., M. T. McCulloch, R. Van Noesik, and D. J. Sinclair. 1999. Corals at their latitudinal limits: laser ablation trace element systematics in Porites from Shirigai Bay, Japan. Earth and Planetary Science Letters 172:221–238.

Fowler, A. J., S. E. Campana, C. M. Jones, and S. R. Thorrold. 1995a. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICP–MS. Canadian Journal of Fisheries and Aquatic Sciences 52:1421–1430.

Fowler, A. J., S. E. Campana, C. M. Jones, and S. R. Thorrold. 1995b. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICP–MS. Canadian Journal of Fisheries and Aquatic Sciences 52:1431–1441.

Gallahar, N. K., and M. J. Kingsford. 1996. Factors influencing Sr/Ca ratios in otoliths of *Girella elevata*: an experimental investigation. Journal of Fish Biology 48:174–186.

Gillanders, B. M., and M. J. Kingsford. 2000. Elemental fingerprints of otoliths of fish may distinguish estuarine "nursery" habitats. Marine Ecology Progress Series 201:273–286.

Hedges, K. L., S. A. Ludsin, and B. J. Fryer. 2004. Effects of ethanol preservation of otolith microchemistry. Journal of Fish Biology 64:923–937.

Ingram, B. L., and P. K. Weber. 1999. Salmon origin in California's Sacramento–San Joaquin River system as determined by otolith strontium isotopic composition. Geology 27:851–854.

Kalish, J. M. 1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph, and otoliths of bearded rock cod *Pseudophycis barbatus*. Marine Ecology Progress Series 74:137–159.

Kraus, R. T., and D. H. Secor. 2004. Incorporation of strontium into otoliths of an estuarine fish. Journal of Experimental Marine Biology and Ecology 302:85–106.

Kraus, R. T., and D. H. Secor. 2005. Application of the nursery role hypothesis to an estuarine fish. Marine Ecology Progress Series 291:301–305.

Martin, G. B., and S. R. Thorrold. 2005. Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xanthurus*. Marine Ecology Progress Series 293:223–232.

Martin, G. B., S. R. Thorrold, and C. M. Jones. 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leistomus xanthurus*). Canadian Journal of Fisheries and Aquatic Sciences 61:34–42.

Miller, J., C. Swanson, and K. S. Poole. 2006. Emergency petition to list the delta smelt (*Hypomesus transpacificus*) as an endangered species under the Endangered Species Act. Center for Biological Diversity, Bay Institute, and Natural Resources Defense Council. Petition to U.S. Fish and Wildlife Service. Available: www.biologicaldiversity.org/swcbd/species/deltasmelt/DS-State-Endangered-Petition-03-08-2006.pdf.

Moyle, P. B. 2002. Inland fishes of California, revised and expanded. University of California Press, Berkeley and Los Angeles.

Moyle, P. B., B. Herbold, D. E. Stevens, and L. W. Miller. 1992. Life history and status of delta smelt in the Sacramento–San Joaquin Estuary, California. Transactions of the American Fisheries Society 121:67–77.

Radtke, R. L., and T. E. Targett. 1984. Rhythmic structural and chemical patterns in otoliths of the Antarctic fish *Notothenia larseni*: their application to age determination. Polar Biology 3:203–210.

Rooker, J. R., D. H. Secor, V. S. Zdanowicz, G. De Metrio, and L. O. Relini. 2003. Identification of Atlantic bluefin tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry. Fisheries Oceanography 12:75–84.

Sadavoy, Y., and K. P. Severin. 1994. Elemental patterns in red hind (*Epinephelus guttatus*) otoliths from Bermuda and Puerto Rico reflect growth rate, not temperature. Canadian Journal of Fisheries and Aquatic Sciences 51:133–141.

Secor, D. H. 1999. Specifying divergent migrations in the concept of stock: the contingent hypothesis. Fisheries Research (Amsterdam) 43:13–34.

Secor, D. H., A. Henderson-Arzapalo, and P. M. Piccoli. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? Journal of Experimental Marine Biology and Ecology 192:15–33.

Secor, D. H., J. R. Rooker, E. Zlokovitz, and V. S. Zdanowicz. 2001. Identification of riverine, estuarine, and coastal contingents of Hudson River striped bass based upon otolith elemental fingerprints. Marine Ecology Progress Series 211:245–253.

SFEI (San Francisco Estuary Institute). 2001. 1999 RMP annual results: Regional Monitoring Program for Trace Substances. San Francisco Estuary Institute, Richmond, California.

Sommer, T., B. Suits, M. Mierzwa, and J. Wilde. 2005. Evaluation of residence time and entrainment using a particle tracking model for the Sacramento–San Joaquin Delta. Report in International Ecological Program synthesis of 2005 work to evaluate the pelagic organism decline (POD) in the upper San Francisco Estuary. Available: www.science.calwater.ca.gov/pdf/workshops/POD/CDFG_POD_Paticle_tracking_model_results.pdf.

SPSS. 2000. SYSTAT 10.0 for Windows: statistics. SPSS, Chicago.

Swanson, C., T. Reid, P. S. Young, and J. J. Cech. 2000. Comparative environmental tolerances of threatened delta smelt (*Hypomesus transpacificus*) and introduced

wakasagi (*H. nipponensis*) in an altered California estuary. Oecologia (Berlin) 123:384–390.

Sweetnam, D. A., and D. E. Stevens. 1993. A status review of the delta smelt (*Hypomesus transpacificus*) in California. Candidate Species Report to the Fish and Game Commission, Sacramento, California.

Thorrold, S. R., C. M. Jones, and S. E. Campana. 1997. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). Limnology and Oceanography 42:102–111.

Thorrold, S. R., C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 1998a. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). Limnology and Oceanography 43:1826–1835.

Thorrold, S. R., C. M. Jones, P. K. Swart, and T. E. Targett. 1998b. Accurate classification of juvenile weakfish (*Cynoscion regalis*) to estuarine nursery areas based on chemical signatures in otoliths. Marine Ecology Progress Series 173:253–265.

Thorrold, S., C. Latkoczy, P. S. Stewart, and C. M. Jones. 2001. Natal homing in a marine fish metapopulation. Science 291:297–299.

Townsend, D. W., R. L. Radtke, S. Corwin, and D. A. Libby. 1992. Strontium : calcium ratios in juvenile Atlantic herring *Clupea harengus* otoliths as a function of water temperature. Journal of Experimental Marine Biology and Ecology 160:131–140.

Trenham, P. C., H. B. Shaffer, and P. B. Moyle. 1998. Biochemical identification and assessment of population subdivision in morphologically similar native and invading smelt species (*Hypomesus*) in the Sacramento–San Joaquin Estuary, California. Transactions of the American Fisheries Society 127:417–424.

USFWS (U.S. Fish and Wildlife Service). 1995. Sacramento–San Joaquin Delta native fishes recovery plan. U.S. Fish and Wildlife Service, Portland, Oregon.

Winer, B. J., D. R. Brown, and K. M. Michels. 1991. Statistical principles on experimental design. McGraw-Hill, New York.